Bio-adrenomedullin as a marker of congestion in patients with new-onset and worsening heart failure

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Abstract

Introduction: Secretion of adrenomedullin (ADM) is stimulated by volume overload to maintain endothelial barrier function, and higher levels of biologically active (bio-) ADM in heart failure are a counteracting response to vascular leakage and tissue oedema. This study aimed to establish the value of plasma bio-ADM as a marker of congestion in patients with worsening heart failure (HF).

Methods: The association of plasma bio-ADM with clinical markers of congestion, as well as its prognostic value was studied in 2,179 patients with new-onset or worsening HF enrolled in BIOSTAT-CHF. Data were validated in a separate cohort of 1,703 patients.

Results: Patient with higher plasma bio-ADM levels were older, had more severe HF and more signs and symptoms of congestion (all *P<0.001*). Amongst 20 biomarkers, bio-ADM was the strongest predictor of a clinical congestion score (r^2=0.198). In multivariable regression analysis, higher bio-ADM was associated with higher BMI, more edema, and higher FGF23. In hierarchical cluster analysis, bio-ADM clustered with edema, orthopnea, rales, hepatomegaly and JVP. Higher bio-ADM was independently associated with impaired uptitration of ACEi/ARBs after 3 months, but not of beta-blockers. Higher bio-ADM levels were independently associated with an increased risk of all-cause mortality and HF hospitalization (HR: 1.16 (1.06-1.27), *P=0.002*, per log increase). Analyses in the validation cohort yielded comparable findings.

Conclusions: Plasma bio-ADM in patients with new-onset and worsening HF is associated with more severe HF and more edema, orthopnea, hepatomegaly and JVP. We therefore postulate bio-ADM as a congestion marker, which might become useful to guide decongestive therapy.

KEYWORDS: bio-ADM; pro-ADM; adrenomedullin; heart failure; congestion

Abbreviations

ACEi	Angiotensin converting enzyme inhibitors
ARB	Angiotensin receptor blockers
Bio-ADM	Biologically active adrenomedullin
BIOSTAT-CHF	A systems BIOlogy Study to Tailored Treatment in Chronic Heart Failure
ET1	Endothelin 1
FGF23	Fibroblast Growth Factor 23
HF	Heart Failure
IL6	Interleukin 6
NT-proBNP	N-terminal pro blood natriuretic peptide
NYHA	New York Heart Association
PENK	Pro-enkephalin

Introduction

Pulmonary and systemic congestion play a major role in symptoms that are associated with worsening heart failure.(1, 2) Consequently, patients who are hospitalized for worsening heart failure with a poor diuretic response and incomplete decongestion at discharge have a greater risk of death and hospital re-admission.(3, 4) Therefore, an easy-to-use surrogate biomarker for the assessment of a patient's congestion status might be of great clinical value.

A promising novel biomarker in this context is the biologically active form of adrenomedullin (bio-ADM), a 52-amino acid ringed vasodilatory peptide hormone that is elevated in acute and chronic heart failure.(5-8) The adrenomedullin (ADM) gene encodes a preprohormone, which after cleavage generates a pro-ADM peptide, which by proteolytic fragmentation becomes a glycine-extended, inactive ADM. This is enzymatically converted to the biologically active ADM. Secretion of ADM has been demonstrated from endothelial cells, cardiac myocytes, vascular smooth muscle cells, and leucocytes. ADM is cleared by neutral endopeptidase, and through binding with its receptors. The most dominant role of ADM is thought to be the regulation of endothelial function, and ADM has been shown to play an essential role in maintaining endothelial barrier function and disruption hereof results in vascular leakage, and systemic and pulmonary edema.(9, 10) ADM expression is stimulated by volume overload and increased plasma ADM reflects excessive fluid overload.(11) In a recent study in patients with acute heart failure, bio-ADM at baseline was associated with more signs of congestion and higher bio-ADM levels after 7 days of decongestive treatment were associated with significant residual congestion at this time point.(7) The findings from this study suggest that bio-ADM might be a potential marker of congestion both at admission and during/after a hospitalization for acute heart failure. In the current study, we aimed to further evaluate clinical and biological factors associated with bio-ADM in patients with worsening and new-onset heart failure, and assess associations with congestion and outcome, in order to gain greater insight in the potential role of bio-ADM in heart failure.

Methods

Study population

We studied plasma bio-ADM in patients enrolled in 'A systems BIOlogy Study to Tailored Treatment in Chronic Heart Failure' (BIOSTAT-CHF), a multicenter, multinational, prospective observational study. In the BIOSTAT-CHF index cohort, 2,516 patients were enrolled with worsening signs and/or symptoms of heart failure from 11 European countries, who were on suboptimal guideline recommended treatment (i.e. ≤50% of target doses of angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARB) and beta-blockers). Patients could be enrolled as in- and outpatients, however the majority of patients were hospitalized for worsening heart failure. A number of patients were admitted or presented to the hospital for "other" reasons, yet developed worsening or new-onset heart failure during hospitalization. Most frequent other reasons were rhythm disturbances, acute coronary syndrome, implantation of a device or uptitration of heart failure medication. Patients should have objective evidence of cardiac dysfunction documented by either left ventricle ejection fraction ≤40% or a blood natriuretic peptide (BNP) level ≥400 pg/ml or N-terminal proBNP (NT-proBNP) ≥2000 pg/ml during screening. During the first three months after enrollment, investigators were expected to optimize treatment of heart failure with ACEi/ARB and beta-blockers, according to the doses indicated in the 2008 European Society of Cardiology Guidelines.(12)

The results were subsequently validated in the BIOSTAT-CHF validation cohort, in which 1,738 patients were recruited from six centers in Scotland, United Kingdom. In summary, in- and outpatients diagnosed with HF with a previous admission for heart failure and maintenance dose of ≥20 mg of furosemide per day were eligible for enrollment. Details on both cohorts have been previously published.(13-16)

All patients provided written informed consent to participate in the study and BIOSTAT-CHF was conducted in concordance with the declaration of Helsinki. The study was also approved by national and local ethics committees.

Study assessments

Plasma bio-ADM was measured at baseline in 2,179 patients in the index cohort and in 1,703 patients in the validation cohort, using an immunoassay (sphingotest® bio-ADM®) developed by sphingotec GmbH (Hennigsdorf, Germany).(8, 17) Included patients were generally comparable to excluded patients for both cohorts (supplementary table 1 and 2). In brief, the bio-ADM immunoassay is a 1-step sandwich chemiluminescence immunoassay based on Acridinium NHS-ester labeling for the detection of human adrenomedullin in unprocessed, neat plasma. It uses 2 mouse monoclonal antibodies, one directed against the midregion and the other directed against the amidated C-terminal moiety of ADM. The assay uses 50 µL of plasma samples/calibrators and 220 µL of labeled detection antibody. Upon storage at room temperature, bio-ADM in EDTA plasma is stable for up to 24 hours, and samples are unaffected by at least up to 4 freeze-thaw cycles. The analytical assay sensitivity is 2 pg/mL. The lower detection limit is 3 pg/mL, and intra- and interassay coefficients were 5-10%, and 4-8% respectively in the above normal measuring range. This assay is highly specific to bio-ADM as it only reacts with the mature amidated C-terminus of ADM, and not to other variants of (pro-)ADM.(17) In healthy subjects, median bio-ADM concentration was 20.7 pg/mL (99th percentile: 43 pg/mL).(18)

A great number of other biomarkers from multiple pathophysiological domains, including markers of inflammation, apoptosis, remodeling, myocyte stress, angiogenesis, endothelial function and renal function were measured. Pro-enkephalin was measured using a sandwich immunoassay (sphingotest® penKid®) targeting PENK A amino-acids 119-159 developed by sphingotec GmbH (Hennigsdorf, Germany). Pro ADM was measured using a sandwich ELISA on a Luminex® platform (Alere Inc., San Diego, CA, USA). Interleukin-6 (IL-6) and endothelin-1 were measured in frozen plasma by Singulex Inc. (Alameda, CA, USA) using high-sensitive single molecule counting (SMC™) technology (RUO, Erenna® Immunoassay System). NT-proBNP was measured using electrochemiluminescence on a Cobas e411 analyzer, using standard methods (Roche Diagnostics

GmbH, Mannheim, Germany). Measurement of other biomarkers was performed as previously described.(15, 19) From all available biomarkers, 20 were selected for these analyses based on their known involvement in volume overload, neurohormonal activation, renal function, and outcome (supplementary table 3).

A congestion score was calculated as the sum of peripheral edema depending on the extent (0 to 1/3 to 2/3 to 1), JVP (0 to 1), and orthopnea (0 to 1) for the index cohort. For the validation cohort, the maximum score is 2, as orthopnea was not routinely collected in this cohort. A modified congestion score, where edema was scored 1 point, if edema extended above the knee, was used in a sensitivity analysis to balance the impact of edema on the congestion score.

Outcomes

The endpoints selected for these analyses were all-cause mortality, cardiovascular mortality, and the combined endpoint of all-cause mortality or first occurrence of HF hospitalization. Cardiovascular mortality was based on the narrative provided for each death by a small committee of cardiologists. Additionally, the association between bio-ADM and therapy optimization for ACEi/ARB and beta-blockers at 3 months was evaluated.

Statistical analysis

Baseline clinical variables and biomarkers were evaluated over tertiles of bio-ADM levels. Frequency (percentage) was used to summarize categorical variables while normally distributed continuous variables were summarized with mean $\pm$ standard deviations (SD) and non-normally distributed continuous variables with median [interquartile range]. Trends over tertiles of bio-ADM were statistically tested with Cochran-Armitage trend test, Jonckheere-Terpstra or a linear regression model for categorical variables, non-normally distributed continuous variables, and normally distributed continuous variables, respectively. Uni- and multivariable linear regression analysis was performed with log transformed bio-ADM as a dependent variable. Transformations were checked

using multifractional polynomials. Multivariable linear regression analysis, including all variables with p<0.10 in univariable analysis were constructed via backward elimination and validated using bootstrap re-sampling with 1,000 replicates. The model was tested for collinearity and checked by plotting residuals. The correlation heat maps and dendrograms were constructed using the ggplot2, reshape2, fastcluster, Hmisc, and sparcl packages in R. Cox proportional hazard regression analysis was performed to examine associations with clinical outcomes. Bio-ADM was investigated as a continuous variable, and by quintiles. Multivariable models were adjusted for an outcome model specifically developed and validated in the BIOSTAT index and validation cohort.(14) The added value of bio-ADM for estimating the risk of poor outcome was assessed by examining gain in Harrell's C-index (a measure of model discrimination, higher values are better), using likelihood ratio tests for nested survival models, and assessment of continuous net reclassification improvement (NRI, a category-independent measure quantifying the degree of improvement in model-based risk estimates obtained by adding a marker to a model). Logistic regression was used to investigate the association between bio-ADM and ACEi/ARB use, and whether target dose was reached. A two-tailed p-value <0.05 was considered statistically significant. All analyses were performed using R: A Language and Environment for Statistical Computing, version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

Median bio-ADM at baseline was 33.8 [22.6-53.9] pg/mL. Baseline characteristics over tertiles of bio-ADM are presented in table 1. Patients with higher bio-ADM levels were older, had a higher BMI, and more severe heart failure (higher New York Heart Association (NYHA) class, higher NT-proBNP levels and more frequently a past heart failure hospitalization), all *P<0.006*. Additionally, higher bio-ADM levels were associated with the presence of signs and symptoms of congestion, lower systolic and

diastolic blood pressure, poorer renal function, and higher levels of biomarkers such as fibroblast growth factor (FGF) 23, pro-enkephalin (PENK), and interleukin (IL) 6, all *P<0.009*. Interestingly, patients with higher bio-ADM levels used higher doses of diuretics (*P<0.001*). After 9 months, patients in the highest tertile were more likely to use loop diuretics (98.2% versus 92.2%, *P=0.018* for first versus third tertile), and used slightly higher doses (40 [40-50] mg versus 60 [40-125] mg of furosemide or equivalent*, P<0.001* for first versus third tertile).

Association between bio-ADM and clinical variables

The result of multivariable linear regression analysis for log bio-ADM is shown in supplementary table 4. The strongest associations with higher log bio-ADM were higher BMI, higher log FGF23, more frequent edema, higher interleukin-6 and endothelin-1 (r^2*=0.580*). Log pro-ADM was significantly associated with log bio-ADM levels yet explained only 30% of the variance of log bio-ADM. Pro-ADM was not significant in a multivariable model for bio-ADM; forcing this variable into the multivariable model increased the r squared significantly to 0.594, *P<0.001*.

Correlation and network analysis

The results of hierarchical clustering of variables and a correlation heat map are presented in figure 1 and 2. The heat map (figure 1) illustrates that bio-ADM levels are most strongly correlated with FGF23 (Spearman's rho: 0.57, *P<0.001*), pro-ADM (Spearman's rho: 0.54, *P<0.001*), GDF-15 (Spearman's rho: 0.49, *P<0.001)*, and the presence of edema (Spearman's rho: 0.43, *P<0.001*). In a hierarchical cluster analysis (figure 2) bio-ADM clustered with edema, as well as orthopnea, rales, hepatomegaly and JVP - all signs of congestion. Interestingly, pro-ADM, did not cluster with bio-ADM, yet clustered with FGF23, GDF15, and NT-proBNP.

Bio-ADM and congestion

Amongst 20 markers, bio-ADM was the strongest variable associated with a higher clinical congestion score (r^2=0.198, *P<0.001*), compared with for instance pro-ADM (r^2=0.120, *P<0.001*), and NT-proBNP (r^2=0.114, *P<0.001*). The modified congestion score yielded similar findings. This was also observed for the presence of edema where bio-ADM had an AUC of 0.768, compared to 0.658 for pro-ADM, and 0.641 for NT-proBNP. Other signs and symptoms of congestion such as JVP and orthopnea yielded comparable findings. In table 2, we show the comparison between associations of bio-ADM and NT-proBNP with clinical markers of congestion. In particular, the association between bio-ADM and edema was stronger than the association between NT-proBNP and edema. The distribution of bio-ADM over quartiles of NT-proBNP (for both cohorts) is plotted in supplementary figure 1. Using ROC analysis, the best cutoff value of bio-ADM to assess congestion (defined as a congestion score > 1) was 34 pg/mL.

Bio-ADM and therapy optimization

Higher levels of log bio-ADM were associated with lower rates of ACEi/ARB and beta-blocker use at baseline (table 1). Also, patients with higher bio-ADM levels less frequently used guideline-recommended doses of ACEi/ARBs and beta-blockers (table 1). Furthermore, higher bio-ADM levels were inversely associated with ACEi/ARB use and dosage after 3 months of uptitration (table 3). This association remained significant after adjustment for body mass index, FGF23, PENK, NT-proBNP, estimated glomerular filtration rate, age, sex, edema and ACE/ARB or beta-blocker use at baseline. In contrast, there was no significant independent association between bio-ADM levels and beta-blocker dosage after 3 months of uptitration.

Bio-ADM and outcomes

During a median follow-up of 21 [15-27] months, 583 (26.7%) patients died and 914 (41.9%) patients experienced the combined endpoint of all-cause mortality or heart failure hospitalization. In univariable Cox regression analysis log bio-ADM was significantly associated with an increased risk of

all-cause mortality (HR 1.79 [1.63-1.97], *P<0.001* per log increase), and the combined endpoint (HR: 1.71 [1.58-1.85], *P<0.001* per log increase). This remained significant for both endpoints after adjustment for the BIOSTAT risk model (table 4), as well as after adjustment for the BIOSTAT risk model with addition of NT-proBNP and the clinical congestion score (HR: 1.19 [1.03-1.36], *P=0.015* per log increase for the combined endpoint). The association of bio-ADM with outcome was also analyzed over quintiles of bio-ADM, where after multivariable adjustment quintile 4 and 5, i.e. the highest bio-ADM levels, remained significantly associated with an increased risk of adverse outcome (table 3). Kaplan Meier curves for all-cause mortality per quintile of bio-ADM are shown in figure 3, illustrating a higher risk over increasing quintiles of bio-ADM (log rank *P<0.001*). Higher levels of bio-ADM were independently associated with an increased risk of cardiovascular mortality (HR 1.27 [1.04-1.56], *P=0.020* per log increase).

When comparing the predictive value of NT-proBNP to bio-ADM for outcome, NT-proBNP was a stronger predictor of all-cause mortality (univariable c-index 0.673 for NT-proBNP versus 0.636 for bio-ADM), as well as the combined endpoint (univariable c-index 0.652 for NT-proBNP versus 0.624 for bio-ADM). Bio-ADM did not improve the net reclassification index on top of the BIOSTAT risk model (including NT-proBNP) for both outcomes (supplementary table 5).

Validation of bio-ADM

The value of bio-ADM in worsening or new-onset heart failure was subsequently validated in a separate cohort of 1,703 heart failure patients. Median bio-ADM was 27.3 [18.0-42.1] pg/mL. Baseline characteristics over tertiles of bio-ADM in the validation cohort are shown in supplementary table 6. The strongest associations with higher bio-ADM levels in multivariable linear regression analysis were observed for edema, higher levels of urea, PENK and higher heart rate (adjusted r^2*=0.357*) (supplementary table 7).

Correlation and network analysis

The heat map (supplementary figure 2) illustrates that bio-ADM levels are most strongly correlated with the clinical congestion score (Spearman's rho: 0.37, *P<0.001*), the presence of edema (Spearman's rho: 0.34, *P<0.001*), and creatinine (Spearman's rho: 0.34, *P<0.001*). Again, the modified congestion score yielded similar findings. In a hierarchical cluster analysis (supplementary figure 3) bio-ADM clustered with NT-proBNP, and diastolic blood pressure. When for the index cohort exactly the same variables as in the validation cohort are entered into a heat map and hierarchical cluster analysis, bio-ADM clustered with edema, JVP, hepatomegaly, and the clinical congestion sore (supplementary figure 4 and 5).

Bio-ADM and outcomes

In the validation cohort during a median follow-up of 21 [12-33] months, 519 (30.5%) patients died and 715 (42.0%) experienced the combined endpoint. Cox regression analyses for (quintiles of) bio-ADM yielded comparable findings to the index cohort (supplementary table 8, supplementary figure 6). Similar to the index cohort, when comparing the prognostic value of bio-ADM to NT-proBNP, NT-proBNP was a stronger predictor of both endpoints.

Discussion

In patients with new-onset and worsening heart failure, higher levels of plasma bio-ADM were strongly associated with more severe heart failure, and signs and symptoms of congestion, and independently associated with an increased risk of all-cause mortality and heart failure hospitalization. Using network analysis tools, such as hierarchical cluster analysis, bio-ADM clustered with markers of congestion. Interestingly, patients with higher bio-ADM levels were less likely to receive target doses of ACEi/ARB after 3 months of uptitration. These findings were externally validated in a separate cohort of patients with heart failure.

Bio-ADM as a marker of congestion

Bio-ADM is vasodilatory peptide hormone that has been shown to play an essential role in maintaining endothelial barrier function, and disruption of this regulatory system results in vascular leakage, and systemic and pulmonary edema.(9) Adrenomedullin acts both intravascular as well as in the interstitium. Intravascular adrenomedullin acts on the endothelial cells and improves vascular integrity and decrease vascular permeability, whereas interstitial adrenomedullin causes vasodilation through its effect on vascular smooth muscle cells.(20) Furthermore, the expression of ADM is stimulated by volume overload, and vice versa increased plasma ADM reflects excessive fluid overload.(11) These elevated levels bio-ADM in heart failure are reflective of a counteracting response to volume overload, as an attempt to limit tissue fluid overload by stabilizing the endothelial barrier function. We recently showed that in patients with acute heart failure, bio-ADM at baseline was strongly associated with signs and symptoms of congestion.(7) Interestingly, higher bio-ADM levels after 7 days of decongestive treatment were associated with significant residual congestion, this was not observed for NT-proBNP. These findings suggest that bio-ADM might be a marker of congestion, that provides additional information over NT-proBNP and could possibly be used to guide decongestive therapy. In this study in patients with new-onset or worsening heart failure slightly lower levels of bio-ADM were observed compared to the previous study in patients with acute heart failure (median bio-ADM 33.8 vs 44.1). This might confirm that in patients with more fluid overload, i.e. acute heart failure, bio-ADM levels are more elevated. In the present study however, bio-ADM levels were increased compared to healthy controls where a median value of 20.7 has been described.(18)

Despite the fact that this study investigated patients with new-onset or worsening heart failure with less fluid overload, the strong association of bio-ADM with congestion is evident. In hierarchical cluster analysis bio-ADM, as the only biomarker, clustered with symptoms of congestion. The slight difference in results between the index and validation cohort, might be due to the fact that patients in the validation cohort were generally less congested and for instance had significantly lower NT-

proBNP levels. Yet, compared to NT-proBNP, bio-ADM was a stronger predictor of a clinical congestion score in both cohorts, and for the presence of edema in particular. The association of bio-ADM with congestion is further supported by the finding that patients with higher bio-ADM levels used higher doses of loop diuretics and were more likely to use loop diuretics after 9 months of follow-up. In addition to an association with congestion markers, bio-ADM also showed a strong association to FGF23. FGF23, is a phosphaturic hormone, that is associated to renin-angiotensin-aldosterone activation and has been postulated as a regulator of sodium hemostasis through upregulation of the sodium-chloride co-transporter in the distal tubule.(21) As such the association of bio-ADM with FGF23 might be indicative of congestion and could also suggest less effective decongestive therapy. Using ROC analyses, the best cut-off for bio-ADM to assess congestion was 34 pg/mL, which is well above the median described in healthy volunteers, and if confirmed in future studies could be used to identify a patient with (residual) congestion. As congestion is notoriously difficult to assess reliably and interobserver variability is considerable, there is a great need for (bio)markers, that aid in reliable assessment of congestion. Our study suggests that bio-ADM is a biomarker that might have unique characteristics in this aspect, and could have clinical utility in a broad range of heart failure patients, i.e. from new-onset to acute heart failure. Future studies, preferentially using more objective measures to assess congestion, such as echography, in addition to physical examination will have to show whether bio-ADM does indeed provide additional information on top of this. Interestingly, patients with higher bio-ADM levels were less well uptitrated with ACEi or ARBs, which may be due to possible residual congestion and diuretic use, or more severe heart failure, hypotension and hemodynamic intolerance of these drugs. The association of bio-ADM with ACEi or ARB uptitration after 3 months was however independent of several markers of heart failure severity, such as NT-proBNP, and baseline use of ACEi or ARB.

Bio-ADM as a marker for clinical outcome

In the current study, we found that higher bio-ADM levels were independently associated with poorer clinical outcome in patients with new-onset and worsening heart failure. This finding confirms previous studies that reported added prognostic value of bio-ADM for shorter-term outcomes.(7, 8, 22) We also found that higher levels of bio-ADM were independently associated with an increased risk of the combined endpoint, which includes both all-cause mortality and heart failure hospitalization. The strong association between higher levels of bio-ADM and a higher risk of hospital (re)admission is of particular interest, since the main cause of heart failure admissions for worsening heart failure is related to congestion. The addition of bio-ADM to a model that included NT-proBNP did however not improve the net reclassification index. Therefore, the main value of bio-ADM might not be in predicting outcome, yet in its role as a congestion marker. As such it would have novel and additive value, compared to more established markers such as troponin or NT-proBNP. Taken together, our findings suggest that bio-ADM might be used to guide (decongestive) therapy, and possibly assess low risk of (re)hospitalization at discharge. Further studies in which bio-ADM is assessed at multiple time points during heart failure hospitalization might be able to shed more light on this.

Adrenomedullin as a therapeutic target

Finally, bio-ADM might be a modifiable risk factor, for instance by administering Adrecizumab, a humanized, monoclonal non-neutralizing antibody against the N-terminus of ADM, that increases plasma concentrations of bio-ADM in a dose-dependent manner.(23) In animal models of systemic inflammation and septic shock, Adrecizumab has shown promising results by improving hemodynamics and renal function.(23, 24) In heart failure, administration of Adrecizumab might result in improved vascular integrity with consequent improvement of tissue congestion and possibly outcomes.(20) Of note, pharmacologic inhibition of neprilysin increased the plasma levels and potentiated the natriuretic and diuretic responses of ADM, when exogenously applied in a dog

model.(25) On that background it could be hypothesized that the beneficial effects of sacubitril/valsartan in heart failure might be partly explained by this mechanism.

Bio-ADM and pro-ADM

In this study, a relatively novel ADM assay was used that assesses biologically active ADM. Until recently ADM activity was measured using a biomarker that assessed an ADM precursor peptide (pro-ADM), which is in contrast to bio-ADM not the biologically active ADM. (17) In this study, pro-ADM explained merely 30% of the variance in bio-ADM levels. Hierarchical clustering showed that pro-ADM did not cluster with bio-ADM or with congestion markers, yet with FGF23 and GDF-15. This suggests that bio-ADM and pro-ADM could provide insight in different underlying pathophysiological processes.

Strengths and limitations

This is the first study to assess the value of bio-ADM in patients with new-onset and worsening heart failure. Strengths of this study are the number of patients enrolled in this cohort, the validation in a separate cohort, as well as the extensive (bio)marker data available. Limitations of this study are the retrospective, observational design, the assessment of bio-ADM at one time point, and the lack of information regarding decongestion or decongestive treatment. Also, the congestion score used in this study is a modified score from the previously published congestion scores due to differences in recording of congestion variables.(26, 27) Furthermore, bio-ADM was particularly strongly associated with easier to assess variables of congestion, i.e. edema and orthopnea, therefore, more objective markers to assess congestion, such as ultrasound, invasive hemodynamic measurements or plasma volume should be studied in subsequent studies. These were unfortunately not available in this dataset. However, at the moment there is no gold standard for the assessment of congestion in heart failure.(28) Based on this study, only associations are described and causality cannot be

proven. (22, 23) Although bio-ADM was associated with clinical outcome in univariable models, there was no strong additive value on top of existing markers of clinical outcome.

Future perspectives

Future studies should assess the value of bio-ADM to guide (decongestive) treatment and might improve assessment of congestion in order to reliably establish euvolemia. Also, therapies aimed at increasing ADM levels, for instance by administration of Adrecizumab or neprilysin inhibitors, could be considered in order to improve outcomes.

Conclusions

Elevated plasma bio-ADM levels in patient with new-onset and worsening HF are associated with typical signs and symptoms of congestion and with an increased risk of mortality and heart failure hospitalization. Bio-ADM might therefore be useful to guide decongestive therapy or might become a target for therapy.

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Figure legends

Figure 1: Biomarker position of bio-ADM depicted in a correlation heat map

Abbreviations: BMI: Body Mass Index; bioADM: biologically active adrenomedullin; DBP: diastolic blood pressure; FGF23: fibroblast growth factor 23; GDF15: growth differentiation factor 15; GFR: glomerular filtration rate; HR: heart rate; JVP: jugular venous pressure; NGAL: neutrophil gelatinase associated lipocalin; NT-proBNP: n terminal pro blood natriuretic peptide; PENK: pro-enkephalin; proADM: pro-adrenomedullin; SBP: systolic blood pressure.

Figure 2: Biomarker position of bio-ADM depicted in hierarchical cluster analysis

Abbreviations: as in figure 1

Figure 3: Kaplan-Meier curve for all-cause mortality for quintiles of bio-ADM in the index cohort

Table 1: baseline characteristics over tertiles of bio-ADM in the index cohort

		Tertile 1	Tertile 2	Tertile 3	P for trend
N =		719	719	741	
bioADM (pg/mL)		19.2 [15.1-22.5]	33.3 [29.1-38.1]	67.2 [53.2-100.6]	
Demographics					
Sex (% Male(n))		76.1 (547)	72.6 (522)	70.9 (525)	0.024
Age (years)		66.9±12.5	69.4±11.7	70.4±11.7	<0.001
Race (% Caucasian(n))		98.9 (711)	98.7 (710)	99.1 (734)	0.756
BMI (kg/m2)		25.8±4.3	27.8±5	29.9±6.3	<0.001
Weight (kg)		76.1±15.6	81.3±16.7	87.7±21	<0.001
Height (kg)		171.3±9.4	170.8±9.2	170.9±9.1	0.402
NYHA class (%(n))					<0.001
	I	3.2 (23)	2.9 (21)	0.1 (1)	
	II	47.4 (341)	38.4 (276)	20.4 (151)	
	III	40.3 (290)	45.1 (324)	57.1 (423)	
	IV	6.5 (47)	11.1 (80)	18.9 (140)	
LVEF (%)		30.5±9.4	31±10.7	31.9±12.2	0.015
HFPEF (%(n))		5.1 (35)	8.3 (53)	10.3 (65)	<0.001
Clinical Profile					
Edema (%(n))		10.7 (60)	22.7 (131)	52.4 (349)	<0.001
Orthopnea (%(n))		23.8 (171)	31.8 (228)	48.2 (356)	<0.001
Rales > 1/3 up lung fields (%(n))		16.8 (48)	19.6 (76)	22 (103)	0.084
Jugular venous pressure (%(n))		21.1 (103)	31.5 (156)	49.4 (239)	<0.001
Hepatomegaly (%(n))		8.5 (61)	12.8 (92)	20.8 (154)	<0.001
Third heart tone (%(n))		10.6 (76)	8 (57)	11.1 (82)	0.731
Clinical congestion score		0 [0-1]	0.67 [0-1.33]	1.67 [0.67-2.33]	<0.001
Systolic Blood Pressure (mmHg)		125.6±22.2	125.4±21.9	122.5±21.9	0.008

Diastolic Blood Pressure (mmHg)	76.1±12.8	75±13.8	72.9±13.1	<0.001
Heart Rate (beats/min)	77±17.7	80.5±20.6	82.6±19.9	<0.001
Hospitalization				
Type of visit (%(n))				<0.001
Scheduled outpatient clinic	33.9 (244)	25.5 (183)	14.3 (106)	
Unscheduled outpatient clinic	6 (43)	3.9 (28)	3.6 (27)	
Inpatient hospitalization	60.1 (432)	70.7 (508)	82.1 (608)	
Reason for visit (%(n))				<0.001
Worsening heart failure	45.1 (324)	51.9 (373)	63.8 (473)	
New-onset heart failure	30.6 (220)	30.5 (219)	26.5 (196)	
Other reason	24.3 (175)	17.7 (127)	9.7 (72)	
Diuretics iv (%(n))	98.7 (310)	97.1 (372)	98.6 (545)	0.918
Inotropics iv (%(n))	10.9 (34)	8.9 (34)	13.2 (73)	0.195
Nitrates iv (%(n))	26.8 (83)	23.2 (89)	17.2 (95)	0.001
Heart Failure History				
Years since first diagnosis	0.4 [0.1-1.3]	3 [0.3-8.8]	3.7 [0.7-7.2]	0.026
Ischemic heart disease (%(n))	56.2 (357)	62.6 (402)	61.6 (405)	0.048
Hypertension (%(n))	54.2 (371)	59.7 (410)	54.9 (389)	0.785
Cardiomyopathy (%(n))	52.8 (349)	38.7 (251)	38.7 (259)	<0.001
Valvular disease (%(n))	37.1 (251)	40.5 (277)	44.4 (310)	0.006
NYHA class prior to decompensation/worsening HF (%(n))				<0.001
I	11 (79)	8.5 (61)	6.1 (45)	
II	51.6 (371)	46.6 (335)	40.4 (299)	
III	24.3 (175)	27.7 (199)	33.7 (250)	
IV	1.9 (14)	4 (29)	4.5 (33)	
Previous HF hospitalization (%(n))	28.4 (204)	29.9 (215)	35.1 (260)	0.005
Medical History				

Hypertension (%(n))	58.3 (419)	65.1 (468)	62.6 (464)	0.091
Atrial fibrillation (%(n))	33.4 (240)	46.2 (332)	57.2 (424)	<0.001
Coronary artery disease (%(n))	38.1 (274)	46.5 (334)	47.1 (349)	0.001
Myocardial infarction (%(n))	32.8 (236)	39.5 (284)	38.3 (284)	0.031
PCI (%(n))	19.1 (137)	22 (158)	21.1 (156)	0.351
CABG (%(n))	11.7 (84)	17.5 (126)	21.7 (161)	<0.001
Pacemaker (%(n))	5.7 (41)	9.5 (68)	7.2 (53)	0.301
ICD (%(n))	5.7 (41)	7.5 (54)	10.4 (77)	0.001
Biventricular pacer (CRT) (%(n))	1.3 (9)	1.3 (9)	3 (22)	0.014
Biventricular Pacer (CRT) and ICD (%(n))	5.4 (39)	6.1 (44)	8.6 (64)	0.014
Diabetes mellitus (%(n))	23.5 (169)	31.6 (227)	41.4 (307)	<0.001
COPD (%(n))	13.1 (94)	18.2 (131)	20.2 (150)	<0.001
Peripheral artery disease (%(n))	7.5 (54)	12.5 (90)	13.6 (101)	<0.001
Stroke (%(n))	8.1 (58)	9.9 (71)	10.4 (77)	0.130
Medication				
ACE-inhibitors or Angiotensin receptor blockers (%(n))	78 (561)	73.2 (526)	64.2 (476)	<0.001
Target dose (%(n))	14.9 (107)	12.4 (89)	10.9 (81)	0.024
Beta-blockers (%(n))	85.1 (612)	83.9 (603)	80.4 (596)	0.017
Target dose (%(n))	4 (29)	6.3 (45)	6.3 (47)	0.055
Loop diuretics (%(n))	99.3 (714)	99.6 (716)	99.6 (738)	0.436
Loop diuretic dose (mg furosemide)	40 [40-62]	40 [40-80]	80 [40-150]	<0.001
Aldosterone antagonists (%(n))	53.5 (385)	53.1 (382)	50.6 (375)	0.259
Digoxin (%(n))	16 (115)	18.9 (136)	20 (148)	0.050
Laboratory				
Hemoglobin (g/dL)	13.7 [12.4-14.8]	13.4 [12.1-14.5]	12.7 [11.4-14]	<0.001
Creatinine (umol/L)	90.2 [76.9-112]	103.8 [87-125.6]	116 [93-153]	0.708
Urea (mmol/L)	9.3 [6.7-14.7]	10.8 [7.5-17.4]	13.5 [8.8-23.8]	<0.001

eGFR (ml/min/1.73m2)	71.1 [53.6-86]	58.6 [44.8-75.5]	49.7 [34.7-67.1]	<0.001
Sodium (mmol/L)	140 [138-142]	140 [137-142]	139 [136-141]	<0.001
Potassium (mmol/L)	4.3 [4-4.6]	4.2 [3.9-4.6]	4.2 [3.8-4.6]	0.003
Calcium (mmol/L)	1.8 [1.5-2.1]	1.8 [1.5-2]	1.7 [1.4-2]	<0.001
Phosphate (mmol/L)	0.9 [0.7-1]	0.8 [0.7-1]	0.8 [0.7-1]	0.611
Albumin (g/L)	34 [29-39]	32 [28-37]	31 [25-35]	<0.001
Iron (umol/L)	10 [6-14]	8.5 [6-12]	6 [4-10]	<0.001
Ferritin (ug/L)	116 [53.2-204.8]	106 [51.5-192]	81 [44-165]	0.057
Transferrin (g/L)	2 [1.6-2.4]	2 [1.6-2.4]	2 [1.5-2.5]	0.890
Aldosterone (pg/mL)	94 [43-187]	93 [44.5-180]	90 [42-211]	0.002
Renin (UI/mL)	68 [23.9-191.7]	89.1 [27.6-229]	125.1 [36.2-374.5]	<0.001
NGAL	50.8 [33.0-80.2]	57.8 [35.7-88.3]	76.3 [45.6-125.4]	<0.001
Interleukin 6 (pg/mL)	3.4 [1.9-6.3]	4.9 [2.7-8.8]	8.6 [4.9-17]	<0.001
Troponin I (ng/L)	10.4 [5.9-22.8]	12.4 [6.6-27.7]	16.3 [9-33.2]	0.785
Endothelin 1 (pg/mL)	4.5 [3.5-5.8]	5 [4-6.7]	6.7 [5.1-9]	<0.001
Fibroblast Growth Factor 23 (RU/mL)	132.3 [90.7-217.5]	201.9 [121.9-401.1]	621.9 [274.6-1745.6]	<0.001
Galectin 3	17.6 [13.0-25.0]	20.3 [15.5-28.0]	26.0 [18.8-36.7]	<0.001
Pro-enkephalin (pmol/L)	73.7 [56.1-97.6]	85.1 [64.5-116.4]	105 [77.2-151.1]	<0.001
Pro-ADM (ng/mL)	0.4 [0.2-0.5]	0.5 [0.3-0.7]	0.8 [0.5-1.3]	<0.001
NT-proBNP (pg/mL)	1826 [825-3959]	2563.5 [1142-5101.8]	4313 [2067-9355.5]	<0.001
GDF15 (pg/mL)	1974 [1306-2912]	2573 [1723.2-3980.2]	4324 [2694-7366]	<0.001

Abbreviations: ACE: angiotensin converting enzyme, bio-ADM: biologically active adrenomedullin, BMI: body mass index, CABG: coronary artery bypass graft, COPD: chronic obstructive pulmonary disease, CRT: chronic resynchronization therapy, eGFR: estimated glomerular filtration rate, GDF: growth differentiation factor, HF: heart failure, HFpEF: heart failure with a preserved ejection fraction, ICD: implantable cardiac defibrillator, LVEF: left ventricular ejection fraction, NT-proBNP: n-terminal pro blood natriuretic peptide, NYHA: New York heart association class, PCI: percutaneous coronary intervention, PENK: pro-enkephalin

Table 2: Comparison of associations with congestion variables between bio-ADM and NT-proBNP

	Bio-ADM		**NT-proBNP**		***P-value (comparison bio-ADM vs NT-proBNP)***
	Correlation coefficient (r)	*P-value*	*Correlation coefficient (r)*	*P-value*	
Edema (above knee)	0.43	<0.001	0.22	<0.001	<0.001
Orthopnea	0.24	<0.001	0.17	<0.001	0.036
JVP	0.29	<0.001	0.28	<0.001	0.767
Third heart sound	0.03	0.168	0.05	0.039	0.630
Hepatomegaly	0.16	<0.001	0.12	<0.001	0.157
Rales	0.19	<0.001	0.20	<0.001	0.587

Presented correlations are Spearman's

Abbreviations: bio-ADM: biologically active adrenomedullin, NT-proBNP: N-terminal pro blood natriuretic peptide

Table 3: bio-ADM and ACEi/ARB or beta blocker use after 3 months of uptitration in the index cohort

	ACEi/ARB use		Target dose		Beta blocker use		Target dose	
Log bio ADM	OR (CI)	P-value	OR (CI)	P-value	OR (CI)	P-value	OR (CI)	P-value
Univariable	0.49 (0.42-0.57)	<0.001	0.67 (0.57-0.78)	<0.001	0.56 (0.47-0.66)	<0.001	0.94 (0.79-1.12)	0.500
Multivariable*	0.60 (0.47-0.79)	<0.001	0.64 (0.47-0.87)	0.005	0.57 (0.42-0.77)	<0.001	0.97 (0.70-1.33)	0.850

* adjusted for BMI, FGF23, PENK, NTproBNP, eGFR, age, sex, edema, and ACE/ARB or beta blocker use at baseline

Abbreviations: ACEi: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, bio-ADM: biologically active adrenomedullin, CI: confidence interval, OR: odds ratio

Table 4: Cox regression analysis for bio-ADM and all-cause mortality, and the combined endpoint in the index cohort

	All-cause mortality				**Combined endpoint**			
	Univariable		Multivariable*		Univariable		Multivariable[#]	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Log bioADM	1.78 (1.62-1.95)	<0.001	1.14 (1.02-1.28)	0.015	1.71 (1.58-1.85)	<0.001	1.16 (1.06-1.27)	0.002
Quintile 1	1.0 (Ref)	Ref	1.0 (Ref)	Ref	1.0 (Ref)	Ref	1.0 (Ref)	Ref
Quintile 2	1.42 (1.02-1.96)	0.037	1.20 (0.87-1.66)	0.275	1.27 (0.99-1.61)	0.062	1.06 (0.83-1.35)	0.670
Quintile 3	1.69 (1.23-2.31)	0.001	1.11 (0.80-1.53)	0.532	1.56 (1.23-1.97)	<0.001	1.11 (0.88-1.41)	0.376
Quintile 4	2.41 (1.79-3.26)	<0.001	1.35 (1.00-1.83)	0.056	2.06 (1.64-2.58)	<0.001	1.23 (0.98-1.56)	0.076
Quintile 5	3.82 (2.87-5.09)	<0.001	1.39 (1.02-1.90)	0.036	3.23 (2.60-4.01)	<0.001	1.33 (1.05-1.68)	0.019

* adjusted for the BIOSTAT risk model for all-cause mortality (age, log blood urea nitrogen (BUN), log NT-proBNP, hemoglobin, and beta-blocker use at baseline)

[#] adjusted for the BIOSTAT risk model for the combined endpoint (age, HF hospitalization in previous year, systolic blood pressure, log NT-proBNP, hemoglobin, high-density lipoprotein, sodium, and beta-blocker use at baseline)

Abbreviations: bio-ADM: biologically active adrenomedullin, CI: confidence interval, HR: hazard ratio.